Native and Acquired Resistance to Tuberculosis*

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THAT a first infection confers increased resistance to a subsequent infection is one of the best established principles in tuberculosis research. However, the immunity acquired is only relative, not absolute as in smallpox. In tuberculosis "the acquired resistance is only a specific increment of the natural resistance."[1] It is incapable of transforming a susceptible species or organ into one that is completely immune.[2] It is desirable, therefore, to consider to some extent the nature of natural resistance.

NATURAL RESISTANCE

It is well known that rats are highly resistant to tuberculosis, yet the tubercle bacillus multiplies readily and persistently within their bodies.[3] However, they develop but slight sensitivity to tuberculin; and although their lesions may be extensive, caseation and softening do not occur.[4] The tubercle bacillus lives almost in symbiosis with the rat. Of a somewhat different nature is the resistance of the chicken to bovine or human bacilli. Bovine tubercle bacilli multiply only to a limited extent in the body of the fowl[5] due to the fact that bovine bacilli multiply meagerly at the body temperature of the chicken. Rich and McKee[6] and Enders and Shaffer[6] have shown that one factor in the immunity of rabbits to Type III pneumococcus is the rapid development of a temperature of 104° to 106°F. (40° to 41.1°C.) in which these pneumococci die.

It is common knowledge that rabbits are susceptible to the bovine bacillus and resistant to the human bacillus. By the use of cultural methods[7] it was found that at first human tubercle bacilli readily multiply in the rabbit. However, although the human bacillus is soon destroyed by an acquired resistance and the animal recovers, the bovine bacillus continues to multiply in the lung and kidney until the rabbit succumbs to the disease. Thus in the slowly progressive disease of tuberculosis it can be seen that what appears on the surface as the native resistance of the rabbit to human type tubercle bacilli really results from a specific resistance acquired during the progress of the disease, for at first no effective opposition to multiplication of this organism exists.

The natural resistance of human beings to tuberculosis bears a certain relationship to the resistance of the rabbit to the human type tubercle bacillus. The vast majority of civilized mankind completely recovers from its primary infection just like the rabbit. There is no race of human beings which is completely immune to tuberculosis. However, the mortality and morbidity from tuberculosis of different races varies widely. Whether these are due to true genotypic, inherited differences in native susceptibility and immunizability or to other factors is still widely debated.

Diehl and von Verschuer[8] studied tuberculosis in identical and fraternal twins. The former have the same genetic determinants; the latter are genetically different. Of thirty-seven identical twins, twenty-six behaved in similar fashion toward tuberculosis. In the remaining identical twins the tuber-

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culosis pursued an unlike course. Thus there was 70 per cent correspondence between the genetic constitution and the behavior toward tuberculosis. On the other hand, among sixty-nine fraternal, i.e., genetically different, twins the tuberculosis was of the same nature in seventeen and of a different character in fifty-two twins; the correspondence in this group was, therefore, only 25 per cent. From these studies the authors conclude that heredity plays a deciding role in tuberculosis. Kallmann and Reisner[9] used the "Twin Family Method" which differs from the twin study previously cited in that not only is the tuberculous morbidity of mono-ovular and bi-ovular twins compared but also that of various sibship groups as well as of the marriage partners of the twin index cases. They found that the chance of developing tuberculosis increased in strict proportion to the degree of blood relationship to a tuberculous index case. They believe that these consistent differences could not be explained by environmental factors.

Plainly, therefore, studies in human beings remain difficult to interpret, for environmental factors cannot be excluded. For this reason Wright and Lewis[10] studied the resistance of guinea pig families that had been inbred for about fourteen generations by brother and sister mating. They found that with subcutaneous injection of a standard dose of tubercle bacilli the difference in longevity was as much as 40 per cent. It is evident from their studies, as critically analyzed by Hill,[11] that one family is genetically endowed with a considerably greater resistance to tuberculosis than the others. In further studies Lewis and Loomis[12] correlated this increased resistance to tuberculosis of guinea pig families with their innate capacity to form antibodies. They found that the most resistant families produced a greater amount of hemolysins and agglutinins when exposed to their antigens.

Lurie[13] has continued these studies in rabbits. By brother and sister inbreeding of rabbit groups for ten to twelve generations, families have been developed which exhibit varying inherited, specific resistance to tuberculosis. This has been determined by exposing succeeding generations of the different rabbit families to artificially infected rabbits or to the inhalation of known numbers of bacilli. It was found after excluding all known environmental factors that resistance to tuberculosis is a function of the genetic constitution of the rabbit. The genetic constitution *per se*[14] determines whether, under given conditions of natural respiratory contagion, rabbits will acquire (1) a rapidly progressive, primary, generalized tuberculosis resembling that seen by Borrel in Singalese troops, (2) a localized, chronic, ulcerative pulmonary phthisis analogous to the "reinfection" type of tuberculosis in white adults, or (3) a disease of a character intermediate between these extremes, as seen in the American Negro.

The fundamental variant of the disease developed by these families is the degree of localization of the infection at the portal of entry. This localization in turn is a function of the rapidity and intensity of the development of local immunity in the primary pulmonary focus. The mononuclears of the most resistant family rapidly acquire an effective capacity to inhibit the growth of tubercle bacilli in their cytoplasm. As a result progress of the disease at the portal of entry is slow. Soon the focus becomes encapsulated and undergoes liquefaction. The bacilli focalized by lymphogenous or hematogenous routes in the lymph nodes or internal organs are prevented from multiplying. Dissemination of the disease takes place chiefly by contiguity or tubular spread of the softened material in which the bacilli multiply. The rate and intensity of development of local immunity in the families of least resistance were low and feeble. The mononuclears did not acquire any considerable capacity to inhibit the growth of the bacilli in their cytoplasm. As a result the primary focus progressed fulminatingly and did not become encapsulated. Dissemination and multiplication of the bacilli took place in the organs focalized by lymphogenous and hematogenous routes.

Recently, by the use of the method of natural quantitative airborne contagion[15,16] through which animals can be exposed to the inhalation of a known number of tubercle bacilli at a single sitting, it was shown that with certain doses of inhaled human type tubercle bacilli and at a given time after infection an extensive tuberculosis pervades the lungs of susceptible rabbits while no tuberculosis at all is found in resistant strains.[17] This difference is due to the fact that the lung of the resistant rabbit soon destroys the human type tubercle bacilli while the lungs of susceptible animals allow them to multiply for a long time. Under these conditions tuberculin sensitivity develops more rapidly in the resistant animals. Thus although the difference in response of susceptible and resistant rabbits to bovine tubercle bacilli is only one of degree, their reaction to the less virulent human organisms results in an all-or-none effect.

These observations suggested that the response of animals to BCG, a strain of tubercle bacilli which never causes progressive disease, might serve as an index to the natural resistance to tuberculosis. It was found that the nodule at the site of intracutaneous inoculation in the resistant rabbit grows rapidly, reaches its peak quickly, tends to ulcerate and heals soon. In the susceptible rabbit this nodule grows slowly, reaches its peak tardily, does not usually ulcerate and heals much later.[18] The BCG multiplies in the skin of the resistant rabbit for a shorter time and is subsequently more rapidly destroyed than in the susceptible rabbit. As a consequence allergic sensitivity to tuberculin develops more rapidly and intensely in the resistant animal. Clearly, whole bacilli cannot sensitize; only on the disintegration of the BCG and the release of its antigens can allergy develop. Likewise antibodies against the tubercle bacillus appear more rapidly and in higher titer in the resistant animal. Thus the varying response of genetically resistant and susceptible rabbits to the highly virulent bovine type bacillus, to the human bacillus of lower virulence for the rabbit and to the BCG of least virulence is characterized by correspondingly graded differences in the uniform reactions of each race. There is no inherent reason why the response of human beings to the intracutaneous inoculation of BCG should not be an accurate index to the native resistance of different individuals to the disease. This seems likely as it is now apparent that man on first infection with the tubercle bacillus, like rabbits of different genetic resistance, may develop either the so-called reinfection type of disease with little or no tracheobronchial involvement or the so-called primary type with extensive disease in the hilar nodes.[19,20]

As to the physiologic basis of this varying inherited local and general resistance, it may be said that in the two most sharply and uniformly contrasting families low skin permeability to particulate matter, rapid and intense development of allergic sensitivity and rapid and high production of antibodies were associated with high resistance; high skin permeability, slow and feeble development of allergic sensitivity and tardy and low production of antibodies were associated with low resistance.

It has been further shown that hereditary resistance to natural airborne contagion of tuberculosis has two phases.[21] Resistance to being naturally attacked by the tubercle bacillus is distinct from resistance to the ensuing disease. One inbred family has little resistance against being attacked by this contagion but great resistance against progress of the ensuing disease. Another inbred family has greater resistance against the engrafting of the infection but little resistance against dissemination of the disease after it has taken root.

Since it was known that estrogen reduces skin permeability[22] and that chorionic gonadotropin, by inducing corpora lutea in the ovaries, increases this permeability,[23] the role of these sex hormones in genetic resistance was investigated.[24] It was found that by subjecting highly inbred susceptible rabbits to estrogen their resistance to an intracutaneous infection could be con-

siderably increased. On the other hand, by exposing resistant animals periodically to chorionic gonadotropin their resistance could be materially diminished. However, the opposite effects of these two hormones resulted not from their different influence on the essential mechanisms of natural resistance, i.e., the rate of multiplication and destruction of tubercle bacilli in the tissues, their allergic irritability or their capacity for antibody production, but through their effects on connective tissue and vascular permeability. Estrogen, by reducing these permeabilities, tends to retard spread of the disease in the body and in a manner unknown protects parenchymatous organs against amyloid degeneration. On the other hand, chorionic gonadotropin via its ovarian consequences increases these permeabilities and enhances dissemination of the infection.

It was noted in these studies that tuberculosis in the rabbit is accompanied with marked hypertrophy of the adrenal cortex. Furthermore, this hypertrophy is proportional, at least to some extent, to the native resistance of the animal.[25] Evidence has also been accumulating which suggests that the adrenal may play an important role in phagocytosis[26] and, via its effect on lymphocytes,[27] in allergic sensitivity and antibody production. It is noteworthy that the adrenals of genetically resistant rabbits are more responsive to stressing stimuli such as cold than those of susceptible animals. An investigation into the role of the adrenals in constitutional resistance is now being actively pursued.

It is clear then that hereditary, constitutional determinants of resistance are numerous and synergestic.

The environmental variable, the intensity of natural contagion,[21] may exercise an important influence both on the incidence and type of disease acquired by these families. At low intensities a small proportion of the least resistant rabbits acquire a fatal tuberculosis while the most resistant family similarly exposed escapes all evidence of disease except for the acquisition of an evanescent tuberculin allergy. High or low intensities of contagion do not change the type of disease acquired by the families of low resistance. It is always fulminating and maximal. In the family of high resistance, however, not only does a high intensity of contagion increase the incidence of the acquired disease but also it modifies the genetically controlled response to the infection to such a degree that the disease is of intermediate type sharing some of the characters of the infection in rabbits of high and low resistance. There is some evidence that diet may affect this genetic resistance.[28]

ACQUIRED RESISTANCE

All investigations on acquired immunity to tuberculosis date from the fundamental observation of Koch[29] who in 1891 described what is known as the Koch phenomenon. It is evident from this phenomenon that to the tuberculous animal the tubercle bacillus is a violent poison; to the normal animal, on the other hand, it is at first almost innocuous. Furthermore and paradoxically, this hypersensitivity to the tubercle bacillus is accompanied with a manifest relative immunity, for although the tissues at the site of local inoculation die, the internal organs are spared for a long time and the regional nodes do not become involved.

Allergy and Immunity. For many years the most widespread opinion has been that allergy, or the capacity of the tissues of the infected animal to react with exaggerated and accelerated acute inflammation to reinfection, is the essential mechanism of the acquired immunity. This was based on the observation that in general there is a parallelism between the two phenomena. Natural and artificial procedures which produce no hypersensitivity to tuberculin do not produce any immunity to tuberculosis. In this country the outstanding work of Trudeau,[30] Baldwin[31] and particularly the investigations of Krause[32] have emphasized the parallelism between allergy and immunity so that one readily believed with Krause that immunity is a function of allergy.

What is the fate of the bacilli of reinfection? In Koch's phenomenon most of the bacilli are extruded from the body together with the slough,[33] but obviously this is a highly unnatural phenomenon. If the site of reinfection does not undergo necrosis, as occurs with small dosage, and if this site is inoculated into susceptible animals, it was found by Römer[34] and by Paterson[35] that the bacilli persist in the tissues in a virulent form.

In 1924 Opie[36] published his important investigations on the Arthus phenomenon. Foreign proteins such as crystalline egg albumin introduced into the skin of a normal rabbit produce slight inflammation, spread widely and soon enter the blood stream. In an animal sensitized to it the introduced protein produces a severe inflammation, remains localized and fails to enter the circulation. Similar observations have been made by Krause and Willis[37,38] in experimental tuberculosis. If the excised lymph nodes draining the site of inoculation of a normal animal are injected into guinea pigs, it can be shown that the bacilli reach these nodes within twenty-four hours. In the reinfected animals it requires two to three weeks for the bacilli of reinfection to pass to these nodes. Since no specific bacteriolysins have been demonstrated in tuberculosis, Krause[39] attributed the fixation of the bacteria at the site of reinfection to the barrier of heightened acute inflammation or allergy. Krause and Peters[40] have also demonstrated that a tuberculous animal reacts with accelerated tubercle formation, and tubercles have been regarded as a factor in limiting the spread of bacilli.

In 1929 Rich and McCordock[41] pointed out that there is really no direct evidence that the death of tissue and the exaggerated acute inflammation which characterize the allergic reaction are in themselves responsible either for the fixation of the bacilli or for inhibition of their multiplication. In a series of interesting investigations Rich and his associates have attempted to separate allergy from immunity. From the standpoint of the present discussion, the most significant observation is that of Rothschild and his associates[42] who rendered guinea pigs hypersensitive to tuberculin by vaccination with an R-1 culture; when allergy was well established, they desensitized these animals by administration of increasing doses of tuberculin until no reaction occurred to the injection of as much as 1 cc. of undiluted tuberculin. These vaccinated and desensitized animals were then given a test dose of virulent tubercle bacilli. Desensitization was continued throughout the course of this disease. It was found that despite the continued absence of allergy these desensitized animals showed a degree of immunity in no way different from guinea pigs similarly immunized but with allergy unaffected by desensitization. These observations have been amply confirmed.

However, Willis and his associates[43] have repeated the experiments of Rothschild[42] but, instead of killing the test animals, allowed them to die. Under these conditions the allergic animals that had been desensitized with tuberculin and maintained in this state for the course of the experiment lived a shorter time, showed more extensive pulmonary disease and contained more tubercle bacilli in their tissues, as demonstrated both by culture and by direct smear, than the guinea pigs that had not been desensitized. It is difficult to state to what extent the results of this treatment with tuberculin are due to the removal of the exaggerated inflammatory responsiveness of the tissues to the tubercle bacillus; it is clear, however, that desensitization with tuberculin, possibly because of the associated malnutrition and adrenocortical exhaustion,[26] interferes with the enhanced capacity of mononuclear phagocytes to destroy or inhibit the growth of tubercle bacilli in them which they acquire as a result of a primary infection. It is noteworthy in this relation that Steinbach[44] demonstrated that sensitized guinea pigs may be protected from tuberculin shock with ascorbic acid which is discharged from the adrenal during the physiologic action of its cortical hormones.

It would seem, therefore, that acute, excessive allergic inflammation is not essential to the operation of immunity. Does inflammation assist in the fixation of bacteria at the site of introduction? In an extensive series of papers Menkin[45] has shown that inflammation has the capacity of fixing *in situ* a variety of substances including bacteria. This, he maintains, is brought about by the coagulated plasma of the exudate and by thrombosed lymphatics that occur at the site of prepared inflammation. It has been shown by Lurie[46] that at the site of tuberculous reinfection in immunized rabbits a greater barrier of fibrin is deposited than in the normal animal. However, with large reinfecting doses incorporated in melted agar and trypan blue the increased lymph flow resulting from the intensified inflammation in the immunized animal brings about a more rapid dissemination of the bacilli, the agar particles and the trypan blue to the draining lymph nodes than in the normal animal, despite the greater barrier in the former. Living tubercle bacilli were demonstrated in the lymph nodes draining the site of reinfection at a time when the regional nodes of the normal animal were still sterile. On the other hand, in guinea pigs, even with large reinfecting doses the bacilli, agar particles and trypan blue are retarded in their dissemination.[47] It was found by Menkin[48] that the fixation of trypan blue at the site of inflammation depends upon the character of the irritant. The powerful necrotizing agent of Staphylococcus aureus produces rapid fixation. Mild irritants, on the other hand, produce only delayed fixation. Now it is well known that guinea pigs develop a far greater sensitivity to tuberculin than do rabbits. In the former the tuberculin reaction often proceeds to necrosis; in the latter this is rarely seen. It is obvious that the inflammation caused by the tubercle bacillus of reinfection in the rabbit is mild and hence is incapable of fixing the bacilli of reinfection. On the other hand, in the guinea pig and especially in the much more highly sensitive human being[49] the exaggerated inflammation may be of such a character as to aid in the fixation. In fact a comparative study of the site of reinfection in rabbits and guinea pigs[50] has revealed significant differences in their character. The lymphatics surrounding the focus of reinfection in the rabbit remain patent and thus apparently permit the passage of tubercle bacilli to the draining lymph nodes. In the guinea pig, however, these same lymph vessels become thrombosed by a delicate and intimate network of fibrin and thus, perhaps, interfere with the ready passage of bacteria.

Rich believes that the fixation of bacilli in the immune animal is a function of the antibody. It has been shown by Mudd[51] and his co-workers that when bacteria are coated with antibody they adhere to each other and to leukocytes with which they come in contact. Rich[52] has shown that in the normal animal virulent bacteria grow dispersed in the tissues. In the immune animal they adhere to each other and therefore grow in clumps in the tissues. They also adhere to fibrin shreds and other tissue elements. This effect of antibody tends to immobilize the bacteria. A similar *in vivo* agglutination has been shown by Lurie[46] to occur in tuberculous reinfection.

Although Rich[53] and his school accept the localizing capacity of an existing inflammation for certain bacteria, they question whether the allergic inflammation in tuberculosis sets in with sufficient rapidity to prevent the dissemination of the bacilli of reinfection. Opie[54] has shown that if carbon particles, staphylococci or tubercle bacilli are suspended in citrated plasma, and if this plasma is coagulated by the addition of calcium, the particles and bacteria become enmeshed in the fibrin network during the process of clot formation while the remaining serum is freed from both. This process may be essential in the mechanism of fixation, the thrombi forming in the lymphatics merely aiding this process. It is likely, therefore, that early in the stage of an allergic inflammation associated with considerable tissue injury the thrombokinase released will quickly clot the exudate poured

out of the blood vessels and thus remove the bacilli from the fluid that drains away from the site of reinfection. That this cell injury occurs within an hour after reinfection would follow from the work of Favour and his associates which is discussed hereafter. The localization of non-specific agar particles and trypan blue at the site of reinfection in sensitized guinea pigs cannot be attributed to antibody action; hence the allergic inflammatory process *per se* mechanically aids fixation. It is concluded, therefore, that an intense allergic inflammation is an aid in the fixation of the bacilli at the site of reinfection.

The fixation of foreign protein in the Arthus phenomenon is in part due to the precipitating effect of the antibody. However, Opie[55] is of the opinion that both in the case of fixation of foreign protein and in the immune reactions associated with inflammation, as in tuberculosis, the relative importance of antibody and inflammation cannot be estimated (both favor fixation), for allergic inflammation brings to the site with greater readiness and in greater amount all the elements of an inflammatory exudate including antibodies, fibrin, granulocytes and macrophages. These processes fix foreign proteins, bacterial products and bacteria themselves at the site of inflammation so that their penetration into the blood stream is retarded.

It is obvious that the antigenic consistency of the tubercle bacillus is complex. Desensitization to allergic inflammation is compatible with immunity if the two are referable to different antigenic components. There is much evidence to suggest that the allergic inflammation is due, in part at least, to the protein as it occurs within the tubercle bacillus in the tuberculous lesions. In experimental tuberculosis in the rabbit Freund and his co-workers[56] found sensitivity to tuberculin subject to considerable variation but the titer of complement fixation remained constant. Enders[57] observed that guinea pigs that had been sensitized with killed tubercle bacilli and that had survived anaphylactic shock to the carbohydrate fraction of the tubercle bacillus show no diminished skin sensitivity to tuberculin. Furthermore, Petroff and his co-workers[58] found that heat-killed avirulent, rough "R," avian tubercle bacilli produce sensitization and some immunity in chickens. Virulent and smooth "S" strains produce more immunity and less sensitization.

Most recently new light has been shed on this phenomenon. Choucroun[59] extracted potent antigenic substances from tubercle bacilli with mineral oil. Middlebrook et al.[60] noted that virulent tubercle bacilli usually grow in skeins or cords due to the fact that on division the daughter cells of a single rod adhere to each other along their long axis. Avirulent bacilli, on the other hand, do not characteristically form such cords but grow in a more or less unoriented fashion. Bloch[61] has continued these studies and has demonstrated that this adhesion of virulent bacilli to each other is due to a lipid on their surface which is soluble in petroleum ether. If virulent bacilli are treated with this hydrocarbon, their viability is not affected but their virulence is reduced. It had previously been found by Allgöwer and Bloch[62] that the motility of phagocytes that had ingested virulent tubercle bacilli is markedly inhibited whereas phagocytes that had ingested avirulent tubercle bacilli are not injured. Treatment of virulent bacilli with petroleum ether robs them of this toxic effect on leukocytes. It is surmised that this lipid may be a determinant of virulence.[63] It is noteworthy that this lipid does not induce tuberculin sensitivity in normal animals nor does it elicit the tuberculin reaction in tuberculous individuals.

Thus sensitization and immunity may be referable to different antigenic agents and no strict parallel need be present between them. It will be shown later that the chief mechanism of immunity is the increased capacity of the mononuclear phagocytes to inhibit the growth of tubercle bacilli. Allergic inflammation is not essential for this process. It is believed, however, that under certain conditions it may aid in the protection.

Nature of Allergy. The profound influence that allergy exerts on the development of the disease warrants its consideration. The tuberculin reaction bears a certain relationship to anaphylactic sensitization to foreign protein, as seen in the Arthus phenomenon. The two conditions, however, are distinct in important respects. Tuberculin sensitivity cannot be induced by the injection of tuberculin. It is conditioned by the presence of tuberculous lesions and varies in intensity with the extent of these;[64] yet Raffel[65] has recently produced delayed tuberculin hypersensitivity by means of the protein plus the wax of the tubercle bacillus. It is noteworthy that this wax induces the formation of epithelioid cells.

Anaphylactic sensitization to tuberculoprotein is independent of such lesions. Baldwin[66] showed that tuberculous guinea pigs occasionally but not uniformly are killed in true anaphylactic shock by the administration of tuberculoprotein. Injection of tuberculoprotein may sensitize normal animals to the tuberculoprotein but not to tuberculin. Austrian[67] demonstrated that sensitivity to the protein of the tubercle bacillus can be transferred to normal animals. There is no conclusive evidence that tuberculin sensitivity can be transferred to a normal animal by humoral substances. Even by the Prausnitz-Küstner technic Coca and Grove[68] failed to transfer this sensitivity. The skin reaction in tuberculin sensitivity appears after a delay of several hours and reaches its maximum development in forty-eight hours. Protein sensitivity of the skin is immediate in appearance and often transitory. Lewis and Seibert[69] and Seibert,[70] by the use of proteins prepared from culture filtrates of tubercle bacilli grown on synthetic media, have demonstrated that such tuberculoproteins are excellent antigens and produce all the classical reactions of anaphylactic sensitization both in rabbits and guinea pigs.

In common with Baldwin[71] and many other investigators Seibert found that rabbits sensitized to the protein of the tubercle bacillus react slightly or not at all to tuberculin. Krause[72] demonstrated that tuberculin sensitivity is associated with some degree of immunity to reinfection. Anaphylactic sensitization to tuberculoprotein, however, develops entirely independently of immunity. Seibert[71] confirmed this observation and has shown that guinea pigs sensitized to tuberculoprotein, far from being protected against infection, actually develop more destructive lesions than the unsensitized controls.

A number of observers[74] have noted that in tuberculin sensitivity the cells are injured on contact with tuberculin. Rich and Lewis[75] clearly demonstrated by tissue culture that the addition of tuberculin to explants of leukocytes or spleen of tuberculous animals inhibits their growth and migration even in the presence of normal serum, whereas to cells derived from normal animals tuberculin is innocuous, even if cultured in tuberculous sera. Aronson[76] confirmed and extended these observations. That circulating antibodies are completely ruled out from these effects and that it is the cells themselves that are sensitized to tuberculin in tuberculous animals has been shown by Moen and Swift[77] who found that this sensitivity to tuberculin is retained by the cells after several transplants in tissue culture.

In 1942 Landsteiner and Chase[78] in studies on experimental drug allergy found that specific hypersensitivity of the delayed type is transferable to normal guinea pigs by means of exudate cells derived from sensitized animals. This was climaxed by the success of Chase[79] in transferring tuberculin sensitivity from sensitized to normal guinea pigs within two to three days after the intravenous or intraperitoneal injection of exudative mononuclear cells, lymphatic or splenic tissue derived from sensitized animals.

With anaphylactic sensitization, on the other hand, and easily demonstrable circulating antibodies, the consensus is that the cells are not injured *in vitro* on addition of the antigen.[80] Meyer and Loewenthal[81] and Aronson[81] have shown that the addition of horse serum to transplants of tissues derived

from guinea pigs sensitized to it has no inhibitory effect on the growth of these cells, even when the cells are cultured in serum containing large amounts of homologous precipitins. Furthermore, in anaphylactic sensitization as in the Arthus phenomenon not only can this sensitization be passively transferred to normal animals by the introduction of the precipitin-containing serum but also, as has been shown by Opie and Furth,[82] inflammation will result in a normal animal by the procedure of reverse anaphylaxis, i.e., by introducing the antigen first and the antibody-containing serum later. In view of this observation it is clear that one need not assume any sensitization of the tissues themselves in the operation of the Arthus phenomenon. In fact, Opie[83] has shown that introduction of the washed precipitate resulting from the interaction of antigen and antibody into normal animals results in inflammation. Doerr[84] has stated that the inflammation in the Arthus phenomenon is not due to the action of the antigen on sensitized tissues but to the action of the antigen-antibody precipitate on the surface of or within normal cells. Rich[85] found that the avascular cornea of a rabbit sensitized to horse serum shows little inflammation on injection of the antigen. However, if the cornea had been previously vascularized by a non-specific irritant, introduction of horse serum into this cornea is followed by severe inflammation, with the greatest injury in the endothelium. From this he concludes that in anaphylaxis the site of sensitization is the endothelium of blood vessels. This interpretation cannot explain the severe inflammation in the reverse, passive Arthus reaction in which tissue sensitization of any kind could not come into question. In a word, tuberculin allergy is a property of the sensitized cells while anaphylactic reactions result from the effects of the interaction of antigen and antibody on cells whose sensitization cannot be demonstrated *in vitro*. An alternative interpretation is that the tuberculin reaction results from the interaction of antigen or tuberculin with antibody which is in intimate union with the cells.

An important contribution to the understanding of the nature of bacterial allergy has been made by Dienes and his associates. If a tuberculous guinea pig is sensitized with horse serum or crystalline egg white, it is found on the sixth day that injection of these antigens into the skin produces large necrotic reactions.[86] This reaction has all the characteristics of the tuberculin as distinguished from the anaphylactic type of sensitivity for it is delayed in appearance, of long duration and is not associated with circulating antibodies. Although these animals react with severe necrosis on injection into the skin of a few hundredths of a milligram of horse serum, they will not be thrown into anaphylactic shock by the intravenous injection of a larger amount of this antigen.[87] Later, when precipitins appear in the blood, the animal can be thrown into anaphylactic shock and the sensitivity can be transferred to a normal animal.

Most recently Raffel[88] has shown that instead of using the whole tubercle bacillus as Dienes did, addition of a purified wax of the bacillus was sufficient to transform the reaction to soluble egg albumin from the immediate anaphylactic type of hypersensitivity, unaccompanied with any evidence of sensitization of the cells to the antigen, into the delayed, tuberculin type of hypersensitivity, with evidence of sensitization of the cells to the egg white as demonstrated by tissue culture and the corneal reaction.

Dienes and Mallory[89] have also shown that the inflammation of the tuberculin type of sensitization is associated in its milder form with a predominance of mononuclears, whereas anaphylaxis and passive sensitization are characterized by an edematous and polymorphonuclear infiltration. Laporte[90] confirmed these observations. Other observers have failed to do so.[91]

Zinsser[92] found that tuberculous guinea pigs first develop tuberculin sensitivity and later anaphylactic sensitization to tuberculoprotein. Dienes maintains that in the

process of sensitization with any antigen, whether of bacterial or other origin, the tissues first develop a sensitization without circulating antibodies, and later the anaphylactic type supervenes with the appearance of antibodies in the blood.

Evidence supporting this view was recently furnished by Chase.[93] It has already been pointed out that he had transferred the delayed types of hypersensitivity to normal animals by administering to them cells derived from sensitized donors. If such normal animals are given six- to eightfold the lymphoid cell mass necessary to confer upon them the delayed type of hypersensitivity, the recipients will show circulating antibodies three to four days after this injection. Thus the lymphoid tissue from sensitized animals at first confers upon normal recipients the delayed type of hypersensitivity and later circulating antibodies.

In tuberculosis the pre-anaphylactic type of sensitization develops to a marked degree. According to Chase's conception, in tuberculin sensitivity and in bacterial allergy in general the antibodies, if any are present, are intimately bound to the cell. Therefore, on contact with the antigen the mononuclear cells undergo injury or multiplication, depending on the concentration of the antigen; hence the predominance of mononuclears in the tuberculin type of sensitivity. The immunity in this stage is chiefly local and operates to circumscribe the antigen. It is noteworthy that Lurie[7] found that when immunity sets in the destruction of tubercle bacilli is much more effective in certain organs than in others, suggesting the role of local factors in the immunity. In fact[50] he has shown that on subcutaneous inoculation of tubercle bacilli the micro-organisms undergo destruction first at the portal of entry. The bacilli in the nearest draining lymph nodes are subjected to destruction next while at the same time the bacilli situated in distant metastatic foci, as in the lung or liver, are still multiplying unhindered. In anaphylactic or passive immunity the circulating antibodies operate to neutralize the antigen that has escaped the barrier of local inflammation. Since tuberculosis in man is largely a local disease of the portal of entry, the lung, it is not surprising that the immunity is chiefly of the local type.

Mechanism of the Tuberculin Reaction. Tuberculin sensitivity can be induced by heat-killed tubercle bacilli. This has been known for a long time, for Prudden and Hoddenpyl[94] demonstrated that killed tubercle bacilli produced typical tuberculous lesions with all their pathologic characteristics. The only difference from the lesions induced by living tubercle bacilli is that the former regress and are absorbed.

That tuberculin sensitivity cannot be transferred by humoral antibodies has been noted previously. However, Chase has transferred this tuberculin sensitivity from allergic to normal animals by means of lymphoid cells and his observations have been amply confirmed.[95] It is noteworthy that the sensitivity thus conferred does not appear in the recipient until two to three days after the administration of the cells, that the passively transferred sensitivity is of short duration and that the cells of the sensitized donor lose this property upon heating to 48°c. for fifteen minutes or on freezing. Is it possible that the transferred cells of the donor elaborate the specific antibody necessary for the tuberculin reaction in the recipient host?

A new and extremely delicate *in vitro* tuberculin test has been developed by Favour and his associates. This depends upon the lytic effect of tuberculin on suspensions of white blood cells of sensitized animals. It was first found that lymphocytes of tuberculous mice suspended in their plasma were lysed by tuberculin.[96] Polymorphonuclears of such mice were not injured by the tuberculoprotein. However, both lymphocytes and polymorphonuclears of tuberculous human subjects were lysed by the tuberculin.[97] It was then observed by this delicate technic that lymphocytes of mice adsorb tuberculin *in vitro* while the polymorphonuclears do not. On the other hand, both types of cells derived from

humans adsorb tuberculin.[98] Furthermore, if white blood cells derived from tuberculous humans are thoroughly washed and suspended in plasma derived from tuberculin-negative individuals, no cytolysis occurs on addition of tuberculin.[99] This can be restored by the addition of plasma from an actively tuberculous individual or by the addition of a heat-labile globulin fraction from such plasma.[100] Moreover, if the complement present in tuberculous plasma is removed by the addition to it of an unrelated interacting antigen-antibody system,[101] no lysis of white cells will occur on addition of tuberculin. This lytic effect of the "decomplemented" plasma can now be restored by the addition of complement derived from plasma of a normal, tuberculin-negative individual. Again, the cytolytic effect of tuberculin on white blood cell suspensions of tuberculous animals is greatly enhanced by the injection of tuberculin intracutaneously forty-eight hours before obtaining the white cells.[102] Finally, by incubating lymphocytes selectively obtained from the blood of actively tuberculous individuals with the plasma of tuberculin-negative individuals, and cytolytically inactive, this treated plasma becomes actively cytolytic to white blood cells of normal individuals upon addition of tuberculin.[103]

In interpreting these observations it must be noted that there is no strict parallel between the cytolytic effect of tuberculin on the *in vitro* suspended cells and the skin reaction of the individuals who furnished the cells and plasma.[104] With this provision in mind and awaiting further confirmation there is a strong suggestion in these studies that the tuberculin reaction may result from the interaction of an antibody present in the plasma of tuberculin-positive individuals, which becomes strongly adsorbed to the white cells, with tuberculoprotein, which also has a strong affinity for the same cells. Their interaction leads to the death of a portion of the cells and to the inflammation which is characteristic of the tuberculin reaction.

Thus the demonstration of Rich that tuberculin is specifically toxic to the tissues of a hypersensitive individual, that the body as a whole and not only the site of the lesion becomes hypersensitive, and the new studies of Chase and those of Favour and his associates would suggest that tuberculin allergy is due to the excretion of minute amounts of antibody from the lesions into the circulation and the rapid anchoring of these agents by the cells. Since the role of this antibody has been demonstrated in the *in vitro* test of suspended white blood cells and not yet in the skin reaction, a final statement as to its significance cannot be made.

Role of Humoral Substances in Tuberculosis Immunity. It has been seen that antibodies with an unusual avidity for cellular adsorption may play a role in tuberculin hypersensitivity. Are antibodies of significance in immunity? Precipitins, agglutinins, complement-fixing antibodies and bacteriotropins have been found in the sera of tuberculous individuals. Passive transfer of these sera confers no immunity on susceptible animals. The sera of highly immunized animals have no bactericidal effect upon virulent tubercle bacilli *in vitro*.

Manwaring and Bronfenbrenner[105] could demonstrate no lysins for tubercle bacilli in the blood or exudates of tuberculous animals; but if tubercle bacilli are incubated with bits of omentum of immune animals, some of the bacilli disappear. The authors attribute this in part to lysis of the bacilli by the fixed peritoneal cells. The peritoneum of normal animals is ineffective. The immunologic significance of the recently demonstrated antibodies[106] in the sera of tuberculous individuals with active disease and their absence in the blood of inactive cases has not yet been elucidated. Thus the antibodies that are found in the body fluids of tuberculous animals have not been shown by *in vitro* methods to play any decisive role in immunity to tuberculosis.

Mechanism of Immunity in Tuberculosis. It is clear from what has been presented thus far that the acute, exaggerated allergic inflammation *per se* is not an indispensable factor in immunity to tuberculosis, nor

have humoral antibodies been demonstrated by *in vitro* methods to play a decisive role in the operation of immunity. *In vitro* studies have also failed to bring evidence that the cells acquire an increased capacity to digest tubercle bacilli as a result of infection. Available information had suggested that the bacilli of reinfection as judged by animal inoculation persist in their virulent form at the site of reinfection. This has led certain investigators to assume that the tissues of the immune animal are changed in some subtle way so as to make them an unfavorable soil for the multiplication of the bacilli[41] or, as Selter[107] and Hedvall[108] maintain, that the tissues of the immune animal become indifferent to the presence of the bacilli and do not react with the formation of lesions.

It was previously stated that rabbits, which are naturally resistant to human tubercle bacilli, overcome this infection not by initially inhibiting the growth of the bacilli but by destroying them almost completely after a brisk preliminary multiplication. Lurie[109] correlated the fate of the living bacilli, as indicated by the number that can be cultured, with the cellular reactions of the body. He found that the immediate polymorphonuclear reaction that follows upon the introduction of tubercle bacilli is greater after the more virulent bovine infection than after the human infection, and more in the lung than in the liver, i.e., they were more abundant with the more virulent strain and in the more susceptible organ. Similar and more extended observations have been made by Long and his associates.[110] This would suggest that the virulent organism is initially more toxic to the tissues than the less virulent bacillus. Moreover, it would also intimate that to the natively more resistant organ the toxic principles such, perhaps, as the surface lipids of the bacillus recently demonstrated by Dubos and Bloch[111] are originally less injurious.

Although polymorphonuclears and mononuclears readily phagocytize the bacilli, as has been shown by Mudd and his coworkers[112] even in the absence of immune serum, the granulocytes do not destroy the bacilli; on the contrary these cells die. These dead polymorphonuclears together with their contained bacilli are then taken up by mononuclear phagocytes. Within these phagocytes the bacilli first multiply without any effective opposition, as indicated by the far larger numbers of bacilli that can be cultured from the lesions at this time and by their accumulation within the cells as observed histologically. Woodruff also made similar observations.[113] It is noteworthy that in this first phase the mononuclears are not injured by the bacilli multiplying in their cytoplasm. Some of the bacilli, however, are destroyed at once. As long as the balance between growth and destruction is in favor of the bacilli, the nodule grows larger by accretion on the periphery of new mononuclears coming either from the blood or by mitosis of pre-existing cells. However, after a varying period and synchronous with development of hypersensitivity to tuberculin[114] and the first stages of caseation, the mononuclear cells begin to assume the structure of epithelioid cells and the bacilli begin to diminish in numbers both histologically and by culture. When the tubercle has matured, most of the bacilli, if they are not of excessive virulence, have disappeared. The rapidity with which epithelioid cells are formed varies with the rate of destruction of the bacilli. In two animals of varying natural resistance to tuberculosis the destruction of the bacilli is more rapid in that animal in which epithelioid cells are formed more quickly. The same is true for different organs in the same individual and even for different parts of the same organ. Likewise, as was shown heretofore, genetically resistant rabbits develop allergic sensitivity more rapidly than susceptible animals because of the more rapid disintegration of the bacilli within their mononuclears; hence the more rapid release of the sensitizing antigens of the bacillus.

It has long been established[40] that one of the chief characteristics of the reinfected animal is the capacity for accelerated forma-

tion of tubercle and its abortive nature. It has been shown by Lurie[115] that in the presence of sufficient residual primary lesion the bacilli of reinfection are quickly destroyed without preliminary multiplication, and this increased destruction is associated with a more rapid mobilization of the mononuclear phagocytes, a more rapid formation of these into nodules and a more accelerated transformation of the phagocyte into mature epithelioid cells. Tubercles and epithelioid cells act, therefore, not merely to hem in and imprison the bacilli as is usually maintained but result from the destruction of bacilli. Thus the most conspicuous and characteristic phase of the tuberculous lesion is the result of the victory of the tissues over the parasite, incomplete though it be, for the destruction of the bacilli is all but complete.[116] Some few bacilli remain and can be cultured from the tissue; hence the virulence of these tissues for susceptible animals and the conclusion that the bacilli are not destroyed in the immune animals. However, even in a disease such as vaccinia with its solid immunity, the virus has been concentrated by cataphoresis by Olitzky and P. H. Long,[117] long after immunity had been established.

This interpretation of the significance of the epithelioid cells and tubercles finds confirmation in the work of Thomas.[118] Sabin and her co-workers[119] in their studies on the role of the phosphatid of the tubercle bacillus in the genesis of the epithelioid cell support this interpretation for they found that as the lipid phagocyted by the monocytes undergoes finer and finer dispersion in their cytoplasm they assume the character of epithelioid cells. Since the liberation of the lipid from the tubercle bacillus and its elaboration by the phagocytes must occur chiefly after the death and disintegration of the bacilli, it is clear that epithelioid cell formation would be associated with the death of the bacilli.

Dienes and Mallory[120] have shown that normal guinea pigs respond with an exudation of polymorphonuclears to the introduction of tubercle bacilli; however, following tuberculous infection and synchronous with the development of hypersensitivity to tuberculin, reinjection of the micro-organism elicits a predominantly mononuclear reaction. Again, Lurie[121] found that the more rapid mobilization of mononuclear phagocytes by the tuberculous and relatively immune animal is an expression of the increased physiologic activity conferred upon these cells by the tuberculous process, for not only do the mononuclear phagocytes accumulate more rapidly in response to the tubercle bacillus but also non-specific irritants such as aleuronat or mineral oil elicit a more rapid mobilization of mononuclear phagocytes in the tuberculous than in the normal animal. This is associated with a demonstrable increase in the rate of amitotic and mitotic division which these cells undergo in the tuberculous as compared with that in the normal animal in their reaction to these non-specific irritants. Furthermore, both *in vivo* and *in vitro*, the mononuclear phagocytes derived from a tuberculous animal exhibit increased phagocytic activity not only for tubercle bacilli but also for unrelated particulate matter such as carbon and collodion particles. This increased physiologic activity of the mononuclear phagocytes of the tuberculous animal is a property of the cells themselves and is independent of the presence of normal or immune serum and, therefore, is not the result of the bacteriotropic action of the immune serum. This increased physiologic activity of the mononuclear phagocytes of the tuberculous, allergic and immune animal, which had previously been inferred, is now definitely demonstrated. This fact perhaps explains the increased capacity of tuberculous animals to form antibodies, as demonstrated by Lewis and Loomis,[122] and the intensification of pre-anaphylactic sensitization in tuberculous animals to antigens in general, as found by Dienes.[86] It is this increased physiologic activity of the mononuclear phagocytes of the tuberculous animal which is perhaps responsible for the increased proteolytic activity of the liver of immunized as compared with normal

rabbits.[123] This same enhancement of physiologic cell activity specifically oriented explains the greater destruction or inhibition of growth of tubercle bacilli in the mononuclears of immunized animals. Lurie[124] caused tubercle bacilli to be phagocytized *in vitro* by mononuclears derived from normal and immunized rabbits in the presence of normal or immune serum and carbon particles. The number of tubercle bacilli ingested by these cells was determined by culture. The normal cells with their load of bacilli and carbon particles were introduced into the anterior chamber of one eye of a normal albino rabbit. Into the other anterior chamber of the same rabbit were introduced the "immune" cells with their ingested tubercle bacilli and particles. After two weeks' growth in this identical *in vivo* environment the two cell types were removed and the number of bacilli they contained was again determined by culture. The cells were identified microscopically by their ingested carbon particles as the albino host had no pigment in his iris cells. It was found that active tuberculosis confers on the mononuclear phagocytes themselves increased bacteriostatic properties against the tubercle bacillus which are independent of the immune body fluids or of the organ environment in which they grow. Qualitative tissue culture studies by Kallos[125] confirmed these quantitative results.

The essential mechanism in immunity to tuberculosis is, therefore, an increased capacity of the mononuclear phagocytes to destroy tubercle bacilli. With small doses of reinfection in a highly immune animal the bacilli are immediately and completely destroyed[115] by the mononuclear phagocytes *in situ*, presumably leaving little if any lipid residue, and hence these cells do not even assume an epithelioid structure. Boquet[126] comes to the same conclusion.

It has been shown by Mudd and his coworkers[127] that promptly following reinfection there is a rise in agglutinating and phagocytosis-promoting antibodies in rabbits that are effectively destroying the bacilli of reinfection. Lurie[46] mixed tubercle bacilli with melted agar and injected the mixture subcutaneously into normal and immunized rabbits. The body fluids readily penetrate the agar which quickly solidifies *in vivo;* the cells invade the agar islands slowly. Under these conditions it has been found that the body fluids of normal animals support the growth of tubercle bacilli, whereas the body fluids of the immune animal that penetrate the acellular agar islands *in vivo* do not support their growth. Furthermore,[50] silk bags were impregnated with a collodion solution of such strength that it permitted the passage of body fluids but prevented the entrance of cells. When such bags containing virulent tubercle bacilli are placed in the peritoneal cavity of normal and immune animals, it can again be shown by culture that in the complete absence of cells the body fluids of the immune animal which penetrate these bags *in vivo* inhibit the growth of the bacilli as compared with the growth of the bacilli in bags placed in normal animals. This is supported by the observation of Thomas and Duran-Reynals[128] who have caused a marked reduction in the rate and extent of development of tuberculous skin lesions in rabbits by mixing the inoculum with an antipolysaccharide serum obtained from a horse treated with living tubercle bacilli.

Furthermore, by the use of the agar focus[46] it can be shown that as Rich[52] had found with the pneumococcus so it is with the tubercle bacillus. In the acellular agar islands of the normal animal the bacilli grow widely dispersed, whereas in the immune animal they are more often found in the form of minute dense clumps, indicating that the surface properties of the bacteria bathed in the immune body fluids tend to make them adhere to each other and reduce their mobility in the tissues. The more pronounced fibrin barrier deposited about the site of reinfection and the thrombosis of adjacent lymph vessels which occurs about the focus in a highly sensitized animal will also aid in fixation of the bacilli. However, even if these growth-inhibiting, immobilizing and fixing properties of the body fluids are

ineffective, the mononuclear phagocytes with their increased capacity to destroy tubercle bacilli are rapidly mobilized and are brought to the site of reinfection and to the invaded lymph nodes where they promptly destroy or effectively inhibit the multiplication of the bacilli of reinfection. It is noteworthy that the impotent polymorphonuclears disappear more rapidly from the site of reinfection than from the site of primary infection.

Thus by appropriate *in vivo* studies it can be shown that acquired resistance in tuberculosis is primarily a function of the cells, but the humoral substances of the immune animal also play a role. It is important to emphasize at this point that the suppression of acute inflammation by treatment with tuberculin does not eliminate the most significant factor in immunity to tuberculosis which is the increased capacity of the mononuclear phagocytes to destroy tubercle bacilli, as in the accelerated formation of tubercle. To the extent that the suppression of allergy diminishes the accumulation of these phagocytes, interferes with their acquired physiologic activities, reduces the thrombokinase released and retards the clotting process and the thrombosis of draining lymph vessels and lowers the concentration of humoral substances at the focus of reinfection, it may be said the reduction of allergy may lower the effectiveness of the immune process. However, the suppression of the acute inflammation, as effected in the reduction of the number of impotent granulocytes brought to this focus, may actually aid in the immune process for, as emphasized by Albert Weil,[129] they act chiefly as agents of dissemination of tubercle bacilli.

As to the essential nature of the change in the mononuclear phagocytes which increases their bactericidal and/or bacteriostatic properties on tubercle bacilli, little is known except that they may be a reflection of the increment of their non-specific physiologic properties, and among these their rate of division and their phagocytic and digestive capacities. As to what is responsible for the specific orientation of these properties to the destruction of tubercle bacilli, nothing is known. It is possible that specific antibodies may be involved which have an avidity for cellular attachment similar to those now being demonstrated as playing a role in tuberculin allergy but developing in response to different antigens such, perhaps, as the surface lipids of tubercle bacilli. Whatever these may be it must be emphasized that the rapidity and intensity of the acquisition of these powers by the phagocytes as a result of exposure to tubercle bacilli are largely determined by the genetic resistance of the host and hence are conditioned by the host's constitutional factors and are unrelated to the parasite. A slight immunization with BCG or even with heat-killed tubercle bacilli will completely protect a rabbit of high genetic resistance against a challenging infection while the same treatment of a susceptible rabbit will only prolong his life for a few months as compared with the survival of an untreated similarly infected littermate.

There is a strange fact that is at variance with all that has been said. Although the tuberculous animal readily destroys the bacilli of reinfection, even if these are highly virulent, the primary lesions may harbor innumerable bacilli even if these are of lower virulence. These primary lesions may gradually extend and kill the animal. Whether this results from an adaptation of the bacilli to the immune state of the animal[130] or to other circumstances is unknown.

It is the persistence of bacilli in old foci which makes the immunity in tuberculosis so variable. Although the bacilli are scanty and gradually tend to die in old caseous foci, separated as they are from the blood stream, during the process of softening which sets in with penetration of blood constituents and often air into the previously immured foci, the residual bacilli here undergo tremendous multiplication in the dead cellular debris. Living phagocytes which penetrate these softening foci die as a result of their allergic sensitivity. The

enormous numbers of living bacilli now spread through preformed channels such as the bronchi and renal tubules or by way of the blood and lymph and overwhelm the existing immunity. The mechanism of softening, which is the most important fact in the mortality of human tuberculosis, is unknown.

Preventive Vaccination against Tuberculosis. Living virulent tubercle bacilli have been cautiously given to man,[131] beginning with single bacilli and gradually increasing. The method had to be abandoned as too dangerous. BCG is a typical tubercle bacillus which multiplies in the body for a short time[114] but is soon destroyed. In the regional lymph nodes isolated organisms persist[132] for a ong time without causing macroscopic lesions or acquiring increased virulence. It produces typical tuberculous lesions, sometimes extensive, including caseation, but with the all important difference that the changes regress and disappear completely. It brings into play the factors tending to immobilize the bacilli of reinfection, inhibit their growth and destroy them with a resulting significant immunity. As long as the organism is propagated according to the directions of Calmette there is no danger of its becoming virulent. The various factors involved in the production of the most desirable vaccine and the mode of its dispensation are now being widely studied.

In the early period of its use, when the BCG was still in the process of attenuation, certain investigators succeeded by special cultural procedures or by animal inoculation in enhancing the growth of the small proportion of still virulent bacilli that were at that time contained in the culture. Since 1929 the attenuation has become so complete that in the last few years evidence has come forth[133] that there is a possibility of the strain becoming too attenuated and hence useless as a vaccinating agent. In any case there is no authentic evidence that in the 1,500,000 infants to whom it was given it has caused a single death. The disaster in Lübeck was apparently due to an error;[134] the same culture sent to Lübeck was innocuous in another laboratory.[135] The protection that it affords is only moderate but apparently sufficient to reduce definitely the tuberculosis mortality of exposed individuals,[136] as indicated by the few carefully controlled studies at hand. The degree of heightened resistance conferred by the BCG is superimposed and determined by the innate resistance of the individual vaccinated. Therefore, it would seem advisable to determine the efficacy of the most desirable preparation of the vaccine on at least two different strains of animals, one of high genetic resistance and one of very low resistance, for both types are present in human populations. Moreover, the increased resistance thus acquired by these two strains may be advantageously gauged by the method of natural quantitative inhalation[16] of single or repeated minimal doses of virulent tubercle bacilli for this simulates both the mode of inception of tuberculosis and the quantities of bacilli involved in natural human infection. The vaccine is best given parenterally as this insures penetration of the vaccine into the tissues and will be indicated by a positive Mantoux reaction. The duration of the immunity conferred is at least five years. It can scarcely be expected that it will protect against marked exogenous reinfection during adult life for even the virulent tubercle bacillus is ineffective under these conditions.

The possibilities of a killed vaccine for prophylactic use in man are still problematic. There is little agreement as to its efficacy in experimental animals. Recently, vaccination with dead tubercle bacilli in combination with other antigens[137] or irritants[138] has yielded promising results in animals. Its use in man has thus far been greatly limited. It is possible that an effective vaccine, derived from an organism of proper antigenic composition, and perhaps in combination with other agents, may still be elaborated.

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